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Scleroglucan gel volume changes in dimethylsulphoxide/water and alkaline solutions are partly caused by polymer chain conformational transitions

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Abstract

The relative equilibrium volume, V/V_0, of swollen scleroglucan gels was determined at temperatures T from 4 to 40°C in aqueous alkaline solutions and binary solvents containing water and dimethylsulphoxide (DMSO), respectively. In some of the experiments scleroglucan was crosslinked using sodium-*meta*-periodate. In others the crosslinks were Schiff bases between the polyaldehyde of scleroglucan and primary amines on small amounts of added chitosan. The V/V_0 showed discontinuous changes at a water weight fraction $W_H = 0.13$, independent of the temperature from 4 to 40°C in DMSO/water solvents. The V/V_0 of the gels decreased when the pH in aqueous solvents was raised above 13. Both these findings coincide with the known strand separation of the triple-helical structure occurring under these solvent conditions. An additional abrupt change in V/V_0 occurred at $W_H = 0.84$ and $T = 20$°C for the binary DMSO/water solvents. This transition was shifted towards lower W_H for increasing temperatures, at $T = 40$°C it occurred at $W_H = 0.56$, which is similar to the temperature effect of the intra-triple-helical conformational transition of scleroglucan. This study indicates that the conformational transitions of the elastically active chains of the polymer network can affect the equilibrium volume of swollen polymer gels. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Scleroglucan gel; Gel swelling; Volume transition; Conformational transition

1. Introduction

Scleroglucan is an extracellular polysaccharide secreted by certain fungi of the genus *sclerotium*. It is a neutral, water soluble polysaccharide consisting of a (1 → 3)-linked β-D-Glc*p* backbone substituted with a (1 → 6)-linked β-D-Glc*p* side chain on every third backbone unit on average (Johnson, Kirkwood, Misaki, Nelson, Scaletti & Smith, 1963). Scleroglucan belongs to the family of (1 → 6) branched (1 → 3)-β-D-glucans where the chemical difference distinguishing the various members mainly is a small variation in the degree of branching (Stone, 1992; Stokke, Elgsæter, Hara, Kitamura & Takeo, 1993). Schizophyllan and lentinan constitute other important examples in this family. X-ray fibre-diffraction data (Bluhm, Deslandes, Marchessault, Perez & Rinaudo, 1982) indicate that scleroglucan adopts a triple-helical structure in the dried state, with the same helical pitch as reported for the (1 → 3)-β-D-Glc*p* homopolymer, curdlan (Chuah, Sarko, Deslandes & Marchessault, 1983). Both hydrodynamic and small-angle X-ray scattering data yield a mass per unit length of the (1 → 3)-β-D-glucans consistent with these polysaccharides being solubilized in aqueous solvents as triple-helical structures (Yanaki and Norisuye, 1983; Gawronski, Aguirre, Conrad, Springer & Stahmann, 1996).

The (1 → 3)-β-D-glucans possess two temperature dependent conformational transitions (Sato, Norisuye & Fujita, 1981; Itou, Teramoto, Matsuo & Suga, 1987; Kitamura and Kuge, 1989) in aqueous solutions. The reversible, intra-triplex structural transition occurs with a mid-point transition temperature $T_m = 5$°C in neutral aqueous solutions. A decrease of the water weight fraction, W_H, in binary aqueous solvents by adding dimethylsulphoxide (DMSO), results in an increase in T_m. This is observed for W_H down to 0.3, followed by a rapid decline in T_m. Addition of alkali, such as NaOH, to the aqueous solvent is another means to raise the transition temperature (Bo, Milas & Rinaudo, 1987; Kitamura et al., 1996). In addition it makes the transition more gradually. The second structural transition involves separation of the triple-helix into fully dispersed

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Table 1
Properties of scleroglucan-periodate and scleraldehyde–chitosan gels

Gel sample designation	C_{Scl} (mg/ml)	C_{Chi} (mg/ml)	D_{Ox} nominal	$r = [NH_2]/[CHO]$	$G'(\omega = 6.2857)$
Scl–Chi1 (Figs.1,5)	8.8	0.45	0.6	0.25	80 ± 20
Scl–Chi2 (Figs.5 and 6)	8.1	0.83	0.6	0.5	550 ± 50
Scl-Per1 (Fig. 2)	18		1		500 ± 60

single chains. The strand-separation transition occurs at $T = 135°C$ in water (Yanaki, Tabata & Kojima, 1985; Kitamura and Kuge, 1989), and is often referred to as denaturation. This process is generally considered to be irreversible (Kitamura and Kuge, 1989). However, regeneration of the overall linear topology of the triple-helical structure was reported following a given annealing protocol for a sample with a molecular weight $M_w = 114 \times 10^3 \text{ g mol}^{-1}$ (Stokke, Elgsæter, Brant & Kitamura, 1992). The apparent irreversibility of the strand-separation transition, at least under certain conditions, therefore appears to be the result of kinetic trapping. Using differential scanning calorimetry, it was found (Kitamura and Kuge, 1989; Kitamura et al., 1996) that other (1 → 3)-β-D-glucans exhibit the same two solvent and temperature dependent conformational transitions both in binary DMSO/water solvents and aqueous alkaline solutions.

The objective of the present study was to determine changes in the polymer gel equilibrium volume associated with conformational transitions in the elastically active chains. Gels with scleroglucan as the elastically active chains were selected as model system. Scleroglucan was crosslinked by using periodate at a relatively high scleroglucan concentration (Christensen, Stokke & Smidsrød, 1999), or by Schiff-base formation between the primary amine group of chitosan and scleraldehyde (Crescenzi, Imbriao, Larez, Dentini & Ciferri, 1995; Guo, Elgsæter & Stokke, 1998). The relative fraction of chitosan in the latter gels is typically less than 5% and always below 10%. This small relative amount of chitosan is expected to influence the equilibrium-swelling properties only as a result of different crosslink densities and not because of changes in the solvent–chitosan interactions. The equilibrium volume of a polymer gel can be very sensitive to the changes in polymer–solvent interaction. Thus, subjecting the elastically active network chains to solvent conditions that are known to induce polymer chain conformational changes, may mediate an additional mechanism yielding discontinuous changes in the equilibrium volume of the gel. Conformation dependent polymer–solvent interaction is one factor expected to contribute to this. Both binary solvents of DMSO and water at different water weight fractions, and aqueous alkaline solvents are employed to induce both the intra-triple-helical and the strand-separation transition of scleroglucan.

2. Materials and methods

2.1. Polysaccharide samples

A scleroglucan with an intrinsic viscosity $[\eta] = 3000$ ml/g and a chitosan with $[\eta] = 1270$ ml/g in 0.1 M NaCl and a degree of acetylation, $F_A = 0.49$ were used and the preparation of the stock solutions were the same as described previously (Guo et al., 1998).

2.2. Preparation of scleroglucan and scleroglucan–chitosan gels

Scleroglucan-periodate gels were prepared (Christensen et al., 1999) by blending appropriate amounts of the scleroglucan stock solution, double distilled water and 0.3 M sodium-*meta*-periodate, $NaIO_4$ (P.a, grade, Merck) freshly dissolved in double distilled water. Gels with different molar ratios between $NaIO_4$ and repeating units of scleroglucan and a total polymer concentration C_{Scl} were prepared.

Scleraldehyde–chitosan gels were prepared according to Guo et al. (1998) using scleraldehyde with nominal 60% aldehyde in the side chains, CS–CHO-60. The scleraldehyde CS–CHO-60–chitosan gels were prepared at pH 7.5–7.6, using selected molar ratios of free amino groups to aldehyde groups, $r = [NH_2]/[CHO]$. Gels were prepared for swelling studies as described, and the storage and loss moduli, G' and G'', were determined in parallel experiments as previously described (Guo, Elgsæter, Christensen & Stokke, 1999).

2.3. Equilibrium volume of swollen scleroglucan gels

Studies of the relative equilibrium volume, V/V_0 of swollen scleroglucan-periodate and scleraldehyde–chitosan gels were carried out by immersing the gel samples in the various solvents. As solvents were used: (a) unbuffered alkaline aqueous solutions with pH ranging from 7.0 to 13.6 (double distilled water adjusted with NaOH, p.a., grade, Merck) or (b) binary solvents of DMSO (p.a., grade Merck) and water at water weight fractions W_H from 0.0 to 1.0. The gels were kept fully immersed in an excess of solvent in sealed flasks. These were stored in a thermostated incubator at selected temperatures for several days until the gels appeared to have reached their equilibrium volume. Hysteresis in the swelling volumes were investigated by first exposing the gels to triplex strand-separation

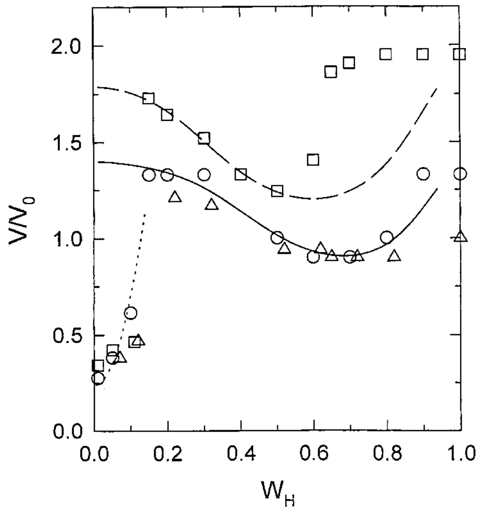

Fig. 1. Experimentally determined swelling ratios V/V_0 of scleraldehyde–chitosan Scl–Chi1 gels versus water weight fraction, W_H, in binary DMSO/water solvents at $T = 4°C$ (△), 20°C (○) and 35°C (□), respectively. The lines (——) and (– – –) depicts model calculations for the equilibrium-swelling data at $T = 35°C$ and $T = 20°C$, respectively. The dotted line for $W_H < 0.14$ is an empirical fit serving only as a guide to the eye.

conditions, i.e., either 0.25 M aqueous NaOH in the pH series, or pure DMSO in the binary solvent series, followed by either decreasing the pH or increasing the W_H, respectively. It was not necessary to take into account the fact that the W_H for the solvent differed from that of the immersed gels because of the solvent volume being much larger than the gel reference volumes. The solvent pH after equilibration of the gels in the alkaline solvents was confirmed experimentally.

The relative equilibrium volumes of the swollen gels were determined as the ratio V/V_0 where V_0 and V are the initial and the final equilibrium volumes of the gels, respectively, according to the previously described procedure (Guo et al., 1999). The data are given as the average of three to five independent determinations, and the relative standard deviations were found to be 2.7–4.5%.

3. Results

3.1. General properties

Scleroglucan-periodate and scleraldehyde–chitosan gels with different polymer concentrations were prepared for the swelling studies (Table 1). The storage modulus of these gels ranged from 80 to 500 Pa (Table 1). The stochiometric ratio $r = [NH_2]/[CHO]$ of the scleroglucan–chitosan gels were 0.25 and 0.5 (Table 1) using the previously determined introduced fraction of aldehyde groups of 0.33 for CS–CHO-60 (Guo et al., 1998).

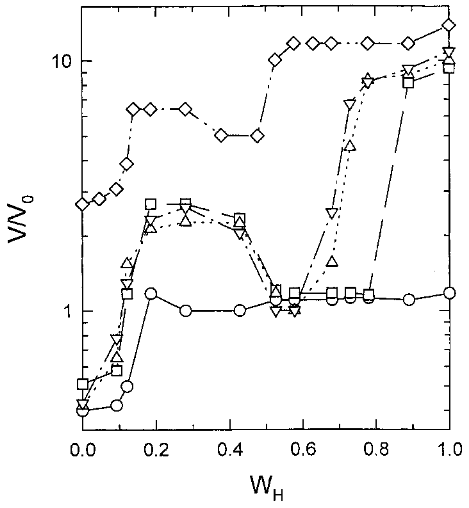

Fig. 2. Experimentally determined swelling ratios V/V_0 of scleroglucan-periodate Scl-Per1 gels versus water weight fraction, W_H, in binary DMSO/water solvents at $T = 4°C$ (○), 20°C (□), 30°C (△), 35°C (▽) and 40°C (◇), respectively.

3.2. Gel equilibrium volume in DMSO/water binary solvent mixtures

Fig. 1 shows the swelling ratio V/V_0 of 8.8 mg/ml scleraldehyde-0.45 mg/ml chitosan gels (Scl–Chi1) as a function of the water weight fraction W_H in DMSO/water solvents at $T = 4$, 20 and 35°C, respectively. The data obtained at $T = 35°C$ show that V/V_0 equals 1.82 at $W_H = 1.0$, and that there is only a minor decrease in V/V_0 when W_H is reduced to 0.7. There is an apparent discontinuous reduction in V/V_0 to 1.35 with a mid-point at $W_H = 0.64$. Following this, V/V_0 increased smoothly upon further reduction of W_H in the range $W_H = 0.6–0.15$. A second and larger relative reduction in V/V_0 was observed at $W_H = 0.15–0.10$ (Fig. 1). Analogous changes in V/V_0 were observed for 18 mg/ml scleroglucan-periodate gels (Scl-Per1) equilibrated in the DMSO/water solvents (Fig. 2).

3.3. Effect of temperature on gel equilibrium volume in DMSO/water solvents

The equilibrium-swelling data show that the discontinuous change of V/V_0 at $W_H = 0.10–0.15$ is almost independent of temperature from 4 to 40°C (Figs. 1 and 2). This contrasts the temperature dependence of the volume transition of the scleroglucan gels observed at $W_H = 0.64$ at $T = 35°C$. The critical solvent concentration W_H^t corresponding to the mid-point for this latter volume transition is reduced to $W_H^t = 0.84$ at $T = 20°C$, and depends linearly on temperatures in the interval $T = 20–40°C$ (Fig. 3). These experimentally determined values of V/V_0 were further compared to the reported conformational transition of such (1 → 3)-β-D-glucans (see Table 2 and Fig. 3).

A further finding worth noting for both the scleraldehyde–chitosan and scleroglucan-periodate gels is the less

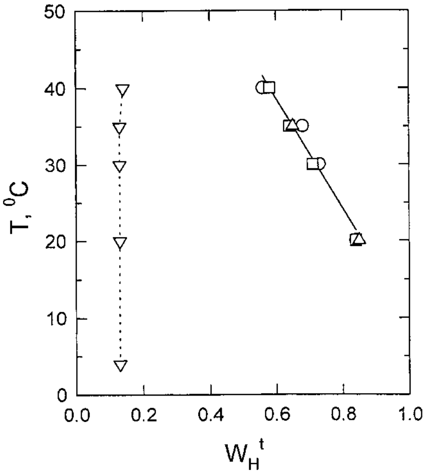

Fig. 3. Comparison of the solvent concentration W_H^t corresponding to the mid-point for the second volume transition of the gels and W_H^t (T) for the intra-triplex conformational transition of (1 → 3)-β-D-glucans determined by calorimetric measurements (Kitamura and Kuge, 1989). Data from Kitamura and Kuge (1989) (□), swelling ratio for scleroglucan-periodate (△) scleroglucan–chitosan gels (○), respectively. The temperature for the mid-point of V/V_0 transition for the volume transition at $W_H \in (0.1–0.15)$ (▽).

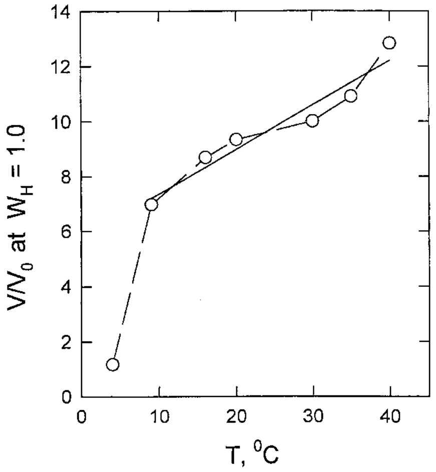

Fig. 4. Experimentally determined V/V_0 of scleroglucan-periodate Scl-Per1 gels in water versus temperature (○). The solid line was obtained using linear regression in the temperature interval 10°C $< T <$ 40°C.

pronounced change in V/V_0 for W_H in the range 0.6–1.0 at 4°C (Figs. 1 and 2) than for the higher temperatures. Fig. 4 shows V/V_0 of swollen scleroglucan-periodate gel in pure water versus temperature. The data show an almost linear dependence on V/V_0 with increasing T in the interval 10–40°C, whereas the observed swelling ratio at $T = 4$°C deviates from this linear dependence (Fig. 4).

3.4. Gel equilibrium volume in alkaline solutions

Fig. 5 shows the swelling ratio of 8.8 mg/ml scleraldehyde–0.45 mg/ml chitosan gels (Scl–Chi1) as a function of pH in aqueous solutions at 20°C. The data show two distinct volume transitions occurring in the alkaline solutions. The swelling ratio of scleroglucan gel remains almost constant ($V/V_0 = 1.52$) over the range of pH = 7.0–11.1, followed by a slight increase in the swelling ratio up to $V/V_0 = 1.94$ from pH = 11.5 to 12.3. A further increase in the pH of the solution led to a sharp volume reduction between pH = 12.3 and 13 where the swelling ratio decreases from $V/V_0 = 1.95$ at pH 12.3 to $V/V_0 = 0.42$ at pH 13.

Table 2
Solvent composition for the second volume transition of scleroglucan gels

Method	Temperature (°C) 4	20	30	35	40
Gel swelling, $W_H^{t\,a}$					
Scl-Per1	Not observed	0.84	0.70	0.64	0.56
Gel swelling, $W_H^{t\,a}$					
Scl–Chi	—	0.85	—	0.65	—
Calorimetry, $W_H^{t\,b}$					
(1 → 3)-β-D-glucans in solution	Not observed	0.84	0.71	0.64	0.56

[a] Determined as the solvent composition for steepest change in the swelling ratio vs. W_H.

[b] Determined from the reported stability diagram of the triple-helical conformations (Kitamura and Kuge, 1989).

Fig. 5 also shows V/V_0 for Scl–Chi1 and Scl–Chi2 with two degrees of crosslinks as a function of pH. The data show that Scl–Chi1 with the lower degree of crosslinking ($r = 0.25$) undergoes two volume transitions, while Scl–Chi2 ($r = 0.5$) only shows one volume transition around pH 13. The volume transition at pH 11.5 is suppressed for the larger crosslink density due to the associated increase in the elastic restoring force contributing to the swelling equilibrium.

The addition of 0.1 M NaCl to the solvent resulted in a reduction of V/V_0 at pH below 10, and almost completely a suppression of the increase in V/V_0 in the pH range of

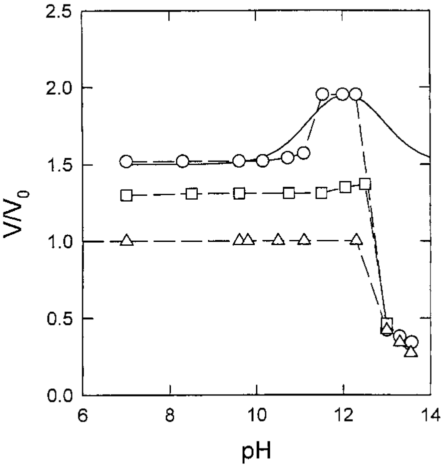

Fig. 5. Experimentally determined swelling ratios V/V_0 of scleroglucan–chitosan Scl–Chi1 gels versus pH at $T = 22$°C (○, □), and Scl–Chi2 gels (△), respectively. The swelling ratios were determined in unbuffered, pH adjusted aqueous solution without added NaCl (○, △) or with 0.1 M NaCl (□). The broken lines are a guide to the eye and the solid lines depict model calculations.

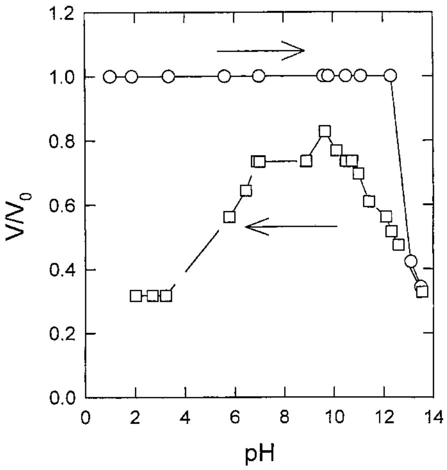

Fig. 6. Experimentally determined swelling ratios V/V_0 of scleroglucan–chitosan Scl–Chi2 gels versus solvent pH at $T = 22°C$. The V/V_0 were determined for pH from 1 to 13 for gels not pre-exposed to high pH (○), and pre-exposed to pH = 13 solution (□).

11–12.5 (Fig. 5). This suggests that the origin of the second volume change is related to the electrostatic interaction, and that the latter would be influenced by the electrolyte concentration.

The potential hysteresis in V/V_0 was investigated for gels with CS–CHO-60, $C_{Scl} = 8.1$ mg/ml, $C_{Chi} = 0.83$ mg/ml prepared at pH 7.6 (the stoichiometric ratio $r = 0.5$). After gelation for 24 h, samples were equilibrated in aqueous solutions containing 0–0.25 M NaOH. The gels first equilibrated in 0.25 M NaOH aqueous solvents were subsequently equilibrated at pH 13.5–2 (Fig. 6). The swelling ratio of the scleroglucan gel after the equilibration in 0.25 M NaOH, first increases with decreasing pH towards a maximum in V/V_0 observed at pH 9–10 followed by a monotonous decline of the swelling ratio with decreasing pH in the solution into the acidic range. This data indicate that the gel swelling may exhibit a hysteresis that depends on the solvent history.

4. Discussion

The swelling behaviour of scleroglucan gels in binary solvents of DMSO/water and aqueous alkaline solutions shares the feature that altered solvent conditions may induce conformational transitions in the scleroglucan chains (Fig. 7). This may affect the equilibrium volume of the swollen crosslinked network for certain solvent conditions. Equally important are the observed differences in the swelling behaviour of these gels in the two classes of solvents. An analysis of gel swelling in aqueous solvent versus pH should therefore include the effects associated with the change from a neutral polymer network to a polyelectrolytic network at pH equal and larger than the pK_a values of the hydroxyl groups. An analysis of the gel equilibrium-swelling volume versus pH was therefore carried out by the use of swelling theories for polyelectrolyte gels (Flory, 1953; Treloar, 1975; Ricka and Tanaka, 1984; Shibayama and Tanaka, 1993; Moe, Skjåk-Bræk, Elgsæter & Smidsrød, 1993). The pH dependent charge density and total ionic strength were included in the description as follows: In the present case where increasing amounts of NaOH was used to adjust pH, a 1:1 electrolyte is considered with ionic strength given by the pH of the solution. The difference in electrolyte concentration, Δc_{tot}, contributing to the Donnan equilibrium proceeds from the equilibrium condition for a 1:1 electrolyte (Tanford, 1961; Moe et al., 1993):

$$c_{Na^+}(g)c_{OH^-}(g) = c_{Na^+}(s)c_{OH^-}(s), \qquad (1)$$

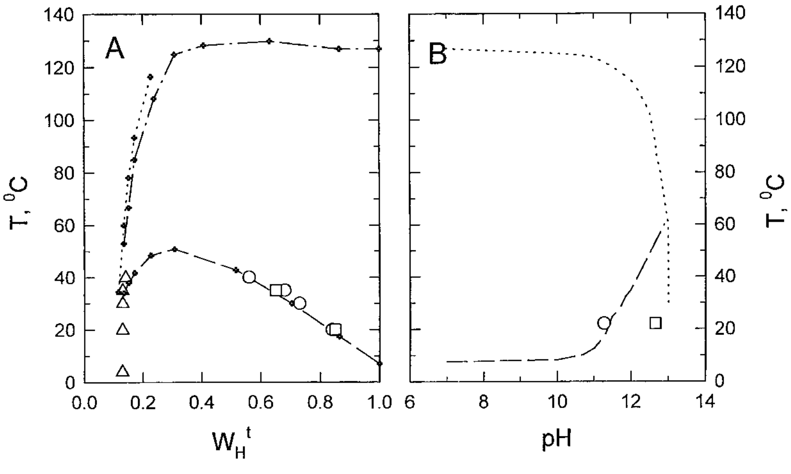

Fig. 7. The phase diagram of the conformational states of schizophyllan in DMSO/Water mixture (a) and alkaline solution (b) where the volume transitions of scleroglucan-periodate and scleraldehyde–chitosan gels have been depicted. (a) Conformational transitions in DMSO/water solvents reported from calorometric studies of schizophyllan sample M-2 (+ ,— — —) and (+ , – – –) and sample U-1 (+ , – · – · –) (Kitamura and Kuge, 1989) and experimental swelling ratio transition data for scleroglucan-periodate (△, ○) and scleraldehyde–chitosan (□) gels. (b) Conformational transition in aqueous alkaline solvents obtained from calorometric studies of schizophyllan sample M-2 (– – –). The line (– – –) represents a rough estimate based on only a limited set of observations for sample M-2. Experimentally determined V/V_0 transition of scleraldehyde–chitosan gels (○, □) corresponding to the transitions depicted with a broken or dotted line, respectively.

where g and s in the parenthesis denote the gel and the sol phase, respectively. The concentrations in the sol phase is given by the added NaOH:

$$c_{\mathrm{Na}^+}(\mathrm{s}) = c_{\mathrm{OH}^-}(\mathrm{s}) = c_{\mathrm{s}} = 10^{\mathrm{pH}-14}, \tag{2}$$

whereas the concentration of Na^+ inside the gel network, $c_{\mathrm{Na}+}(\mathrm{g})$, is larger than the OH^- concentration, c_{OH^-} (g), by the amount required to neutralise the fixed charges on the polymer network:

$$c_{\mathrm{Na}^+}(\mathrm{g}) = c_{\mathrm{OH}^-}(\mathrm{g}) + \rho\phi/M_2. \tag{3}$$

In Eq. (3), ρ equals the density of the dry polymer network and ϕ the volume fraction of polymer. M_2 is the molar mass of polymer per unit charge, which depends on pH because of the dissociation of hydroxyl groups:

$$M_2 = M_{2,0}(1 + 10^{\mathrm{p}K_\mathrm{a}-\mathrm{p}H}), \tag{4}$$

where $M_{2,0}$ is the molar mass per ionizable (hydroxyl) group, and $\mathrm{p}K_\mathrm{a}$ the dissociation constant of the hydroxyl groups. Note also that increasing the charge density, indicated by reducing M_2, above a critical value will lead to partial neutralisation by immobilised counterions as described by the Manning condensation theory (Manning, 1978). This effect results in an effective maximum fixed charge density on the polymer.

Employing Eqs. (1) and (4) in the theory of swelling of polyelectrolyte gels accounts for the observed maximum in V/V_0 at pH around 12, whereas the observed reduced V/V_0 for $\mathrm{pH} > 12.5$ could not be accounted for (Fig. 5). The continuous line is an example calculated using $G' = 100$ Pa (close to the experimentally determined G', Table 1), $M_{2,0} = 1280\ \mathrm{g\ mol}^{-1}$ (repeating unit of scleroglucan), $\rho = 1.61\ \mathrm{g\ cm}^{-3}$ and molar volume of the solvent $V_1 = 0.018\ \mathrm{l\ mol}^{-1}$. The Flory–Huggins interaction parameter in this calculation was $\chi = 0.44$, and the maximum extension ratio $\lambda_{\max} = 1.34$. The latter parameter was used in the inverse Langevin function to include finite extensibility of the non-Gaussian chains (Treloar, 1975; Schröder and Oppermann, 1996). The $\mathrm{p}K_\mathrm{a} = 12.1$ of the model calculation shown is slightly lower than the reported $\mathrm{p}K_\mathrm{a} = 12.35$ for hydroxyl groups (Rendleman, 1973) merely to adjust the maximum of V/V_0 in the model to the experimental one. Including the effects of the supporting electrolyte in the analysis also reproduced the experimental suppression of the maximum in V/V_0 at about pH 12 observed for the solvent series which did not include 0.1 M NaCl. Despite the non-Gaussian nature of the network chains being included only to a limited extent, these semi-quantitative calculations show that the maximum in V/V_0 can be accounted for by gel swelling driven mainly by a lack of effective screening of the $-\mathrm{O}^-$ groups resulting from dissociation of the –OH hydroxyls in this pH range. The subsequent decrease in V/V_0 arises from an increased screening of the bound charges at larger electrolyte concentrations (higher pH).

The almost discontinuous reduction in V/V_0 observed for pH greater than 13, and the fact that V/V_0 is nearly independent of the crosslinking density above pH 13 are not accounted for in this consideration. pH values greater than 12.5 are reported to induce strand separation of (1 → 3)-β-D-glucans (Kitamura et al., 1996; Gawronski et al., 1996), and hence, suggested to be the main molecular mechanism underlying the reduced V/V_0 in the present data.

The equilibrium-swelling properties of polyelectrolyte gels in binary solvents in the approximation of a Gaussian network have been analysed by Iwatsubo, Masuoka & Mizoguchi (1994) and Iwatsubo, Ogasawara, Yamasaki, Masuoka & Mizoguchi (1995). This theory was adopted here for semi-quantitative calculations to compare with the experimental swelling data in binary DMSO/water solvents. The finite extensibility of the non-Gaussian network chains was incorporated using the inverse Langevin approximation (Treloar, 1975; Schröder and Oppermann, 1996), and the network chains were assumed to be neutral because scleroglucan constituted the main fraction of the material. Model calculations using G' close to the experimentally determined $G' = 80 \pm 20$ Pa (Table 1), and $\lambda_{\max}$ similar to that for the swelling versus pH, were able to account semi-quantitatively for the swelling data for $0.14 < W_\mathrm{H} < 0.85$ at $T = 20°\mathrm{C}$ and $0.14 < W_\mathrm{H} < 0.55$ for $T = 35°\mathrm{C}$. The Flory–Huggins type interaction parameter between the two solvent components, χ_{12}, and the two interaction parameters between the polymer and the solvent components, are difficult to determine. The reported (Cowie and Toporowski, 1961) exothermic reaction taking place when DMSO is mixed with water, possibly because of the formation of complexes with a DMSO:H_2O molar ratio of 2:1, suggests a negative χ_{12}. The molecular mechanism underlying the increased V/V_0 observed both for the scleroglucan-periodate and scleraldehyde–chitosan gels around $W_\mathrm{H} = 0.3$ could originate from preferential solvation of the polysaccharide chains by one of the two solvent components. Straub and Brant (1980) suggested that there is an excess solvation of monosaccharides by water molecules in the mixture of range $W_\mathrm{H} > 0.34$ and excess solvation of DMSO molecules for $W_\mathrm{H} < 0.34$. When W_H is decreased from 0.5 to 0.15, the swelling ratio is slightly increased. This may arise from the preferential solvation of scleroglucan by DMSO in this range of W_H which is substantiated by the report of Sato, Norisuye & Fujita (1983a) stating that DMSO preferentially solvates such (1 → 3)-β-D-glucans in the range $W_\mathrm{H} < 0.4$.

The data for the scleroglucan-periodate gels differed from those of the scleraldehyde–chitosan gels by showing a sharper increase in V/V_0 for W_H being reduced from 0.5 to 0.4. The temperature dependence of the plateau of V/V_0 for W_H 0.2–0.4 showed only a slight increase for T from 20 to 35°C, but increased from 2.7 to 6.5 upon further increase in T to 40°C (Fig. 2).

The nearly constant V/V_0 for W_H below 0.2 in the model calculations differ from the experimental observations of a discontinuous volume transition occurring for $W_H \cong 0.10$–0.15 (Figs. 1 and 2). This discontinuity in V/V_0 is almost independent of temperature from 4 to 40°C, and is consistent with the reported temperature insensitivity of the triple-helical strand-separation transition at $W_H = 0.13$ (Figs. 3 and 7). These two findings suggest that the discontinuous change in V/V_0 occurring at $W_H = 0.13$ is driven by scleroglucan strand-separation transition similar to that at pH 12.5 for the aqueous solution series. A reduction of the mean-square end-to-end distance associated with the strand separation is one effect expected to contribute to the observed reduction in V/V_0. The reported decrease in the intrinsic viscosity of (1 → 3)-β-D-glucans in the same range of DMSO (Sato, Sakurai, Norisuye & Fujita, 1983b) corraborate this interpretation despite the topological constraints imposed in the gel state compared to that in solution. Additionally, differences in the interaction parameters and a change in the crosslink density may also affect the V/V_0 in the same direction as that observed experimentally.

The experimentally observed discontinuous change in V/V_0 with a mid-point at $W_H^t = 0.64$ at $T = 30$°C (Figs. 1 and 2), was not reproduced in the model calculations. This discontinuity in V/V_0 appears to be related to the reported mid-point of the intra-triple-helical conformational transition. The temperature corresponding to maximum observed excess specific heat, T_m, at $W_H = 0.7032$ characterising this transition for (1 → 3)-β-D-glucans in solution was reported to equal $T_m = 30$°C (Kitamura and Kuge, 1989). This agrees favourably with the gel swelling data. The additional finding that the temperature dependence of the critical solvent concentration W_H^t parallels that reported of the conformational transition (Figs. 3 and 7), also points in the direction of the conformational transition being involved. A prerequisite for the intra-triple-helical conformational transition being important in this discontinuity in V/V_0 is that the interaction parameters differ in the two conformational states.

The lack of complete reversal of V/V_0 observed for reduction of pH following high pH denaturation (Fig. 6), indicates that the flexible single coiled chains do not recombine to the same structure as that before denaturation. The renaturation process being initiated at random along the chains can envisage this and the initial linear triplex topology are not regenerated. Compared to the gels not being denatured, this state may differ in crosslink density, and a fraction of topologically trapped single-stranded segments having a different solvent interaction parameter than that of the triplex state. Changes in both these parameters are expected to affect the equilibrium-swelling ratio.

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